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Article type : O - Original Article

DIRECT ACTING ANTIVIRAL AGENTS-BASED REGIMEN FOR HCV RECURRENCE AFTER COMBINED LIVER-KIDNEY TRANSPLANTATION: RESULTS FROM THE ANRS CO23 CUPILT STUDY

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Abbreviations:

AE: Adverse events

CKD: Chronic kidney disease

DAAs: Direct-acting antiviral agents

DCV: Daclatasvir

EOT: End of treatment

GFR: Glomerular filtration rate

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

IMS: Immunosuppressive

KT: Kidney transplantation

LLOQ: Lower limit of quantification

LT: Liver transplantation

MDRD: Modified Diet in Renal Disease

MELD: Model for End-stage Liver Disease

MMF: Mycophenolate Mofetil

PEG-IFN: pegylated interferon

RBV: Ribavirin

SAE: Serious adverse events

SOF: Sofosbuvir

SVR: Sustained virological response

TBC: Trough blood concentration

W: Week

Abstract

HCV infection is associated with reduced patient survival following combined liver-kidney transplantation (LKT). The aim of this study was to assess the efficacy and safety of second generation direct active antiviral (DAAs) in this difficult-to-treat population. The ANRS CO23 CUPILT study is a prospective cohort including transplant recipients with recurrent HCV treated with DAAs. The present work focused on recipients with recurrent HCV following LKT. The study population included 23 patients. All patients received at least one NS5B inhibitor (Sofosbuvir) in their antiviral regimen an average of 90 months after LKT. Ninety six percent of recipients achieved a SVR at week 12 (SVR12). In terms of tolerance, 39% of recipients presented with at least one serious adverse event. None of the patients experienced acute rejection during therapy and there were no deaths during follow-up. The glomerular filtration rate (GFR) decreased significantly from baseline to the end of therapy. However, this study did not show that the decline in GFR persisted over time or that it was directly related to DAAs. The DAAs-based-regimen is well tolerated with excellent results in terms of efficacy. It will become the gold standard for the treatment of recurrent HCV following LKT.

Introduction

Severe hepatitis C virus (HCV)-related liver diseases (decompensated cirrhosis and especially hepatocellular carcinoma) remain a major indication for liver transplantation (LT) in most centres worldwide (1-2). In France, more than 20% of LT candidates have HCV

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infection (3). HCV is also a well-known cause of chronic kidney disease (CKD) such as type I membrano-proliferative glomerulonephritis, usually associated with type II mixed cryoglobulinemia potentially leading to end-stage renal disease requiring hemodialysis and/or kidney transplantation (4-5). HCV-related concomitant liver and kidney diseases are a specific indication for simultaneous liver-kidney transplantation (LKT). The use of the Model of End Stage Disease (MELD) score for liver allocation policies in the 2000s has resulted in a significant increase in the number of LKT in the United States. Indeed, 8.2% of all LTs performed in the USA in 2014 were LKT compared to 2.5% in 2001 (OPTN data http://optn.transplant.hrsa.gov). However, HCV recurrence always occurs on the graft in patients with a positive viral load which has represented a challenge because the progression of fibrosis is accelerated compared non-transplant patients (6-7). Recurrence of HCV following isolated LT or KT negatively influences both graft and patient survival (5-10).

Until recently, antiviral treatment of transplant recipients with recurrent HCV was limited to the use of first generation protease inhibitors (boceprevir-telaprevir) following LT increased the SVR at 24 weeks to 47% in genotype 1 patients, but was associated with a high rate of serious adverse events (SAEs), and concerns about interactions with immunosuppressive drugs (11-12). Since 2013, the use of second-generation direct acting antivirals (DAAs) has provided major progress in isolated LT recipients with recurrent HCV whatever the stage of liver fibrosis and in previously “difficult-to-treat” patients with fibrosing cholestatic hepatitis (13-15). A SVR 12 weeks after treatment was obtained in more than 90% of cases in all subpopulations with fewer adverse events than in previous studies. Recent published studies have shown that second generation oral DAAs also effectively treated HCV infection following isolated kidney transplantation with a low rate of treatment-related side effects (16-18). These reports suggest that the outcome of LKT recipients treated with DAAs should be favourable. However existing data is limited to a recent observational retrospective study including 7 LKT patients (18). This is a specific population that has received two grafts and is obviously more likely to present with organ dysfunction and/or have a higher rate of side effects than patients with LT or KT alone. Thus, a

prospective study is needed to provide robust data on the safety and efficacy of DAAs these patients.

The ANRS C023 “Compassionate use of Protease Inhibitors in viral C Liver Transplantation” (CUPILT) study is a prospective multicenter cohort study sponsored and funded by ANRS (France REcherche Nord&Sud Sida-hiv Hépatites) that has enrolled isolated LT and LKT recipients with recurrent HCV who have been treated with second generation DAAs. The aim of the present study was to assess the efficacy and tolerance of second generation DAAs-based regimens for recurrent HCV after LKT.

Patients and Methods

Patients and study design

The ANRS C023 “Compassionate Use of Protease Inhibitors in viral C Liver Transplantation” (CUPILT) study is a multicentre prospective cohort performed in 25 French and Belgian LT centers (ClinicalTrials.gov number NCT01944527). Included patients 1) received a liver graft combined or not with a kidney graft, 2) developed recurrent HCV whatever the stage of fibrosis, 3) were treated with second-generation DAAs, and 4) gave their written informed consent. The stage of fibrosis was determined at enrolment based on a histological assessment (according to the METAVIR scoring system (19) and/or elastometry (such as F3 ≥9.5 kPa and F4 ≥14.5kPa). The protocol was performed in accordance with the Declaration of Helsinki and French laws on biomedical research, and was approved by the "South Mediterranean Ethics Committee” (France). Exclusion criteria were patients under 18 years old and pregnancy.

From October 2013 to December 2015, 699 patients with recurrent HCV were included in the cohort. The present study evaluated LKT patients receiving DAAs who were followed-up for at least 12 weeks after treatment discontinuation. This study is observational so the type of treatment, dosing of drugs and duration of treatment were at the discretion of

each investigator. However, the CUPILT Scientific Committee (Appendix 1) issued treatment recommendations. Patients received either: Sofosbuvir (SOF) and Daclatasvir (DCV); or SOF and Ledipasvir (LEDI); or SOF and Simeprevir (SIM); or SOF and PEG. If RBV was used the dose was adjusted according to body weight, potential RBV-related hematological toxicity and renal function in recipients. Treatment duration was initially planned for 12 or 24 weeks, but the investigators are allowed to extend this period if they considered it to be clinically necessary. Trough blood concentrations (TBC) of calcineurin inhibitors were monitored during treatment. During antiviral treatment, modification in the dose of calcineurin and mTOR inhibitors or MMF was performed at the investigator's discretion.

Efficacy assessments

In this study, plasma HCV RNA levels were quantified using the Abbott Real Time HCV PCR assay (lower limit of quantification (LLOQ) of 12 IU/mL, Abbott Diagnostics®, USA) or COBAS AmpliPrep® or COBAS TaqMan® (LLOQ of 15 IU/mL, Roche Molecular Systems, Pleasanton, California). HCV RNA was monitored at baseline, during scheduled visits throughout treatment (1, 2, 3, 4, 6, 8, 12, and if applicable 16, 20 and 24 weeks) and then at follow-up week 4 (FUW4) and FUW12 after the end of treatment (EOT).

The primary endpoint was the proportion of patients who achieved undetectable HCV RNA levels or an SVR at FUW12 after treatment discontinuation (SVR12). Secondary endpoints included viral kinetics, and on-treatment (W4, W8, and W12), end-of-treatment (EOT) and FUW4, FUW12 and FUW 24 response rates. Data on survival and liver and kidney functions were reported at FUW 48. Virological failures were also reported. Viral breakthrough and relapse were defined as plasma HCV RNA levels above the LLOQ after achieving a level below the LLOQ during treatment and EOT, respectively.

Safety assessments

Data were collected for the following adverse events: serious adverse events (SAE), as defined in Supplemental Appendix 2, clinical and laboratory grade 3 or 4 adverse events (assessed using the INSERM-ANRS scale to score the severity of adverse events, and given in Supplemental Appendix 3) and adverse events of any grade related to neutrophils, platelets, prothrombin values, bilirubin, creatinine, haemoglobin or infections. In LKT transplant recipients, particular attention was paid to the kinetics of renal function based on the glomerular filtration rate (GFR) before, during and after DAAs therapy. GFR was estimated using the Modified Diet in Renal Disease (MDRD)-6 and the CKD-EPI equations (20-21).

Decisions to reduce, interrupt, or discontinue RBV because of toxic effects were based on the drug manufacturer's labelling. The investigators were encouraged to manage all AE according to guidelines issued by the French Association for the Study of the Liver (AFEF) (22).

Statistical Analysis

Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA) and R 3.3.0. In case of a non-normal distribution, continuous variables were expressed by median and inter-quartile ranges and in case of normal distributions, continuous variables were expressed by means and standard deviations. Categorical variables were expressed by the number of patients and percentages. Differences in baseline characteristics between the two groups, depicted by RBV use, were evaluated using the Wilcoxon-Mann-Whitney test in case of non-normal distributions or using one-way analysis of variance in case of normal distributions for continuous data and the chi-square test or Fisher's exact test for categorical data. Repeated-measure analysis of variance was used to test for changes over time in continuous variables. When the time was significant,

Tukey's honest difference (HSD) was used for multiple comparisons. $P<0.05$ was considered to be significant in all statistical tests and 95% confidence intervals (CI)

Results

Baseline demographic and clinical characteristics

Twenty-three LKT recipients treated with DAAs were included in the present study between October 2013 and December 2015. The baseline characteristics of these recipients are presented in Table 1. Recipients were mainly men (73.9%), median age 58 years old [52-64]. LKT was performed for kidney transplant candidates with cirrhosis in 10 cases (43.5%), for liver transplant candidates with severe chronic renal dysfunction in 11 cases (47.8%), for metabolic disease (primary hyperoxaluria) in one case and for calcineurin inhibitors nephrotoxicity in one case. Recipients had the following HCV genotypes: genotype 1a in 5 cases (21.7%); 1b in 9 cases (39.1%), 3 in 5 cases (21.7%) and 4 in 3 cases (13.0%). Fifteen recipients (65.2%) were treatment-naïve; previously treated recipients were non-responders (26.0%). Nine recipients (40.9%) developed extensive fibrosis or cirrhosis on the graft and 3 had fibrosing cholestatic hepatitis (13.6%). The median time between LT and the beginning of treatment was 82.0 months [25.9-126.7]. All the recipients received at least one NS5B inhibitor (Sofosbuvir) in their antiviral regimen. Antiviral therapy regimens are provided in table 2. Only ten recipients (43.5%) received RBV. The treatment duration was 12, 24, 28 and 32 weeks for 10, 11, 1 and 1 patient, respectively. There was no statistical difference for demographic and clinical characteristics between recipients who did or did not receive RBV.

Treatment efficacy

Table 3 shows the virological response during and after DAAs therapy in the entire population and in relation to the use of RBV. Figure 1 shows the kinetics of median HCV viral load according to RBV use. By W4 of treatment, HCV RNA levels had fallen below the LLOQ in 14 patients (63.6%), 6 patients who received RBV (66.7%) and 8 who did not (61.5%) with no statistical difference between the two regimens. Undetectable HCV-RNA was achieved after a median of 4 weeks [2–8]. For the primary endpoint, 10/10 patients treated with RBV (100%) and 12/13 without (92.3%) had a SVR12 (Figure 2). One virological relapse was observed at FUW4 in a F3 recipient treated with SOF (400 mg x3/week) and SIM (150 mg/d) for 12 weeks. This patient had HCV genotype 3a with baseline viral load of 7.27 log.

Safety

Adverse events were common and occurred in 20 recipients (87.0%), although most were mild to moderate. The most common adverse event was anemia (Table 4). As expected, anemia was more frequent in recipients treated with RBV (80.0% vs 23.1% without ($p=0.0123$). In recipients treated with RBV, the dose of RBV was reduced then discontinued for 2 recipients, only reduced for 4 recipients and discontinued for 1 recipient. A serious AE was reported in 9 recipients (39.1%): severe infection in 3 recipients (13.0%) with favourable outcomes (CMV-induced colitis, pneumonia, septicaemia related to urinary tract infection), and 1 case of haematuria, basocellular carcinoma, stroke, acute leg ischemia, acute kidney failure, and anaemia/leukopenia. None of the recipients died during the study or experienced acute cellular or humoral rejection.

Immunosuppression dose modifications were necessary in 11 recipients (47.8% of cases) including changes in tacrolimus dosage in 6 patients between baseline and W4 and in 1 patient between W4 and EOT, as well as changes in cyclosporine dosage in 4 recipients between W4 and EOT. No changes in MMF were required. Median tacrolimus serum

concentrations were not different between baseline and EOT (3.6 [3.2-6] ng/mL vs 4.2 [3.4-5.3] ng/mL, p=0.864).

Kinetics of renal function

In the overall population there was a moderate but significant decrease in eGFR values between baseline and EOT (from 52.5±29.2 to 46.9±28.5 mL/min; p=0.027) and between baseline and FUW12 (52.5±29.2 to 47.2±27 mL/min; p=0.0039). To determine this unexpected effect, we evaluated the potential cause of impaired eGFR. First, the daily dose of calcineurin inhibitors was not significantly modified between baseline and EOT in tacrolimus (1.9 mg/day [1.5-2.0] vs 2.1 mg/day [2-2], p=0.13), or cyclosporine (100 mg/day [100-125] vs 100 mg/day [100-100], p=0.25). Second, the kinetics of eGFR were analysed 12 months before, during and after DAAs therapy in relation to the use of RBV (Figure 3). The kinetics were different before anti-viral treatment in relation to RBV use (RBV+: +1.36±2.15 mL/min/month vs RBV-: -0.56±1.04 mL/min/month, p=0.005) but not during (-0.27±1.47 mL/min/month vs -0.29±0.59 mL/min/month, p=0.9) and after DAA therapy (-0.42±1.32 mL/min/month vs -0.16±1.17 mL/min/month, p=0.6) (figure 4). The variation in kinetics in the entire population before, during and after therapy was not statistically significant (mean GFR variation +0.28±1.85 mL/min/month vs -0.28±1.04 vs -0.27 ± 1.21, p=0.35). Finally, we determined the individual slope of GFR before, during and after DAAs therapy (Figure 5). The slope of most of the recipients with a positive slope before DAAs (83.3%) worsened > 50% during treatment. However, after therapy the slope was worse > 50% in only 3 recipients (30%) and the slope reversed in most. The slope was only worsened in 4 recipients with a negative slope before DAAs after DAA therapy.

Follow up

All LKT patients except one were alive at FUW48. One patient died between FUW24 and FUW48 from de novo skin carcinoma. At FUW48 the median [IQR] liver function tests were all within normal ranges; AST 20 IU/L [14-24], ALT 16 IU/L [11-24], γGT 34 IU/L [22-61] and

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total bilirubin 8 µmol/L [7-14] while median [IQR] renal function was; creatinine 140 µmol/L [100-338], eGFR MDRD 47 mL/min [13.3-72.1] and eGFR CKD-EPI 47.5 mL/min [13-73.7]. None of the recipients experienced acute cellular or humoral rejection at FUW48. The recipient who developed acute kidney failure required a kidney transplantation at FUW84. This patient was a 49-year-old woman who underwent retransplantation for recurrent HCV cirrhosis on the first liver graft. A kidney transplantation was associated with the liver transplantation because of chronic kidney dysfunction related to severe calcineurin inhibitor nephrotoxicity. Baseline eGFR was 19.4 mL/min. Acute kidney failure occurred early on day 7 when eGFR reached 17.6 mL/min requiring hemodialysis. The patient was successfully treated with SOF+DCV+RBV for 24 weeks. Hemodialysis was necessary throughout the study and until kidney retransplantation.

Discussion

Treating liver transplant recipients with an all-oral, interferon-free, antiviral regimen is now the standard of care (13-15). To our knowledge, this is the largest series of LKT patients with recurrent HCV treated with second generation DAAs for 12 or 24 weeks whatever the genotype or stage of fibrosis at baseline. The results of this study, with an excellent SVR12 rate of 95.7% on intention-to treat analysis, show the efficacy of DAAs in treating LKT. Our results were especially important in the most difficult to treat patients, who represented more than half of the population, such as those with extensive fibrosis or cirrhosis or fibrosing cholestatic hepatitis (13). The combination therapy was well tolerated, although SAE rates occurred in 39.1%. None of the patients died and there were no reported episodes of rejection.

There are two major reasons to eradicate HCV in LKT patients; first, recurrent HCV following isolated LT is associated with significantly reduced long-term graft and patient survival compared to other indications because of universal HCV reinfection of the graft and

accelerated progression of fibrosis in immuno-compromised patients (6-8). Also HCV can generate and promote CKD in the general population and is associated with lower survival in hemodialized and kidney transplanted patients (4-5, 9-10). For example, a meta-analysis of observational studies including more than 133 000 KT recipients showed a relative risk of graft loss and mortality of 1.76 and 1.85 respectively in patients with anti-HCV-positive serology (23). Thus, HCV eradication in these populations should be associated with lower rates of liver decompensation, CKD or retransplantation with an expected outcome following LKT that is similar to other indications of LKT without HCV.

The development of DAAs has provided major progress in the therapeutic management of patients with HCV. Indeed, one major finding is the high rate of eradication in all patient subgroups whatever the stage of fibrosis. Overall, SVR rates following solid organ transplantation are now similar to those observed in immunocompetent patients. Earlier antiviral treatments first-generation protease inhibitors (boceprevir or telaprevir) were associated with high rates of potentially life-threatening SAEs and acute cellular rejection was the most severe AE in daily clinical practice (11-12). IFN-free regimens with DAAs have several advantages in these cases: most available drugs have no or limited pharmacological impact on trough blood concentrations of calcineurine inhibitors, and most DAAs, unlike first generation protease inhibitors, do not have potent drug-drug interactions. Indeed, in the recent study by Dumortier *et al*, only minimal dose modifications of immunosuppressants were necessary and no significant over or under-dosages were observed (15). To date, NS5B inhibitors such as SOF have usually been the backbone of IFN-free antiviral regimens. Despite their renal metabolism, these inhibitors can be used in patients with CKD without safety concerns. Safety was carefully investigated in our study population. In our cohort, the SAE reached 39%, most frequently anemia followed by infection with favourable outcomes. As expected, RBV use was associated with lower hemoglobin levels, a higher rate of anemia, EPO and red blood cells transfusions as well as the need for dose reduction. RBV administration is still a question because there was no significant difference in SVR between

patients who did or did not receive RBV, while the tolerance was markedly worse in the former.

Finally, there was a small but significant reduction in GFR during treatment (from 52.5 to 46.9 mL/min. between baseline and EOT) observed in the study cohort. No correlation was found with RBV use or daily doses of calcineurin inhibitors. Moreover, no significant variations in mean GFR were found between the periods before, during and after therapy. Individual analysis of the slope of GFR showed that most recipients who presented a worsening of the slope had a negative slope before starting therapy. When worsening occurred during therapy this was reversed after therapy stopped. Finally, the GFR slope only worsened in a minority of recipients (n=3) after therapy. Thus, our study did not show that the decline in GFR persisted over time and was directly related to DAAs. These positive results are similar to those in the CORAL-I and SOLAR-1 trials reporting DAA use following LT. In the CORAL I study, mean creatinine clearance was 90.5 ml per minute at baseline and 85.9 ml per minute at week 24. None of the patients had a creatinine clearance of less than 50 ml per minute during treatment (25). In the SOLAR study there was no specific assessment of GFR kinetics. However, only one patient experienced an episode of acute renal failure in the cohort of 229 transplanted patients (26). Further studies are needed to investigate the predictive factors of renal impairment during antiviral therapy. Other studies from the ANRS CO23 CUPILT cohort investigating pharmacokinetic changes and renal function are ongoing. A final report of the entire cohort will be also published.

In conclusion, the combination SOF ± NS5a inhibitor ± RBV for 12 or 24 weeks resulted in an SVR12 rate of 95.7k% in LKT recipients. Overall, this regimen was safe and well tolerated. DAAs regimens could become the gold standard for the treatment of recurrent HCV following LKT, allowing earlier treatment of patients before the development of graft fibrosis and CKD. This approach could help eradicate HCV infection in this specific population at high risk of both severe liver and kidney diseases and life threatening events, in whom treatment guidelines are still awaited.

Acknowledgments

We would like to thank all the patients and their families, and all the co-investigators of the CUPILT study in France and Belgium for their support and for making this study feasible. Our particular thanks go to Laurent Alric, Teresa Maria Antonini, Ellada Ayvazyan, Eleonora De Martin, Djamel Elaribi, Laure Esposito, Stéphanie Faure, Joelle Guitard, Stéphanie Haïm Boukobza, Laurence Lavayssière, Sophie Metivier, Gilles Pelletier, Bruno Roche, Lionel Rostaing, Faouzi Saliba, Rodolphe Sobesky, Maria Grazia Tateo, Rodolphe Anty who participated actively in the study. We also acknowledge the assistance provided by the clinical research associates and data-manager: Mustapha Ahmim, Neila Bouchard, Alain Caro, Hélène Danjou, Selma El Andaloussi, Anne Laligant, Khellaf Ouamara, Julie Raimon, Régine Thierry and Aurélie Veislinger. Finally, we thank all the staff at the ANRS (France REcherche Nord&Sud Sida-hiv Hépatites) who were responsible for this study, and notably Professor Jean-François Delfraissy, Inga Bertucci, Alexandra Rohel, Alpha Diallo and Ventzislava Petrov-Sanchez.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. Sebastien Dharancy has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Chiesi and Novartis. Audrey Coilly has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Novartis, Merck Sharp & Dohme and Abbvie. Nassim Kamar has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Merck Sharp & Dohme, Novartis, Alexion, Fresenius, Amgen and Roche. Vincent Leroy has been a clinical investigator, speaker and/or consultant for Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Merck Sharp & Dohme, and Roche. Pauline Houssel-Debry has

been speaker and/or consultant for Astellas, Novartis and Gilead Sciences. Jérome Dumortier has been a clinical investigator, speaker and/or consultant for Astellas, Gilead Sciences, Janssen Pharmaceuticals, Novartis and Roche. Vincent Di Martino has been a clinical investigator, speaker and/or consultant for Abbvie, Bristol-Myers Squibb, Gilead Sciences, Jansen Pharmaceuticals, Merck Sharp & Dohme. Victor De Ledinghen has been a clinical investigator, speaker and/or consultant for Abbvie, Bristol-Myers Squibb, Gilead Sciences, Jansen Pharmaceuticals, Merck Sharp & Dohme. Christine Silvain has been a clinical investigator, speaker for Abbvie, Bristol-Myers Squibb, Gilead Sciences, Jansen Pharmaceuticals, Astellas. Danielle Botta-Fridlund has been a clinical investigator, speaker and/or consultant for Abbvie, Bristol-Myers Squibb, Janssen, Gilead and Roche. Jean-Charles Duclos-Vallée has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Abbvie, Novartis and Roche. Georges-Philippe Pageaux has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Merck Sharp & Dohme, Novartis and Roche. The other authors have no conflicts of interest to disclose.

Figure Legends

Figure 1: Virological kinetics of HCV viral load during DAA therapy according to RBV use (results are expressed as median and interquartile range)

Figure 2: Virological responses according to treatment duration and RBV use

Figure 3: Kinetics of GFR before, during and after therapy according to the use of RBV

Figure 4: GFR variation before during and after DAAs according to the use of RBV

Figure 5: Evaluation of the GFR slope before, during and after DAAs

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Table 1: Baseline demographics and diseases characteristics

	n=23
Gender (male)	17 (73.9%)
Age (years)	58 [52-64]
Body mass index (kg/m^2)	22±3.6
Diabetes	11 (47.8%)
Cardiovascular disease	5 (21.7%)
Arterial hypertension	13 (56.5%)
HIV co-infection	2 (8.7%)
Indication for LT	
Cirrhosis	11 (47.8%)
HCC	5 (21.7%)
Re-LT	6 (26.1%)
Other	1 (4.3%)
Indication for KT	
Hemodialysis	10 (43.5%)
CKD	11 (47.8%)
Other (metabolic)	2 (8.7%)
Delay after LKT (months)	82.0 [25.9-126.7]
Immunosuppressive drugs	
Cyclosporine	9 (39.1%)
Tacrolimus	14 (60.9%)
Sirolimus	1 (4.3%)
MMF	10 (43.5%)
Previous therapy post-LKT	
Treatment-naïve	15 (65.2%)
PEG+RBV	7 (30.4%)
PEG+RBV+BOC	1 (4.3%)
Previous course of HCV therapy	
Treatment-naïve	15 (65.2%)
Relapse	2 (8.7%)
Non responders	6 (26.0%)
HCV genotype	
1	15 (65.2%)

3	5 (21.7%)
4	3 (13.0%)
HCV viral load (log10IU/mL)	6.41 [5.76-6.92]
Fibrosis stage at baseline	
F0-F2	10 (45.5%)
F3-F4	9 (40.9%)
fibrosing cholestatic hepatitis	3 (13.6%)
Cryoglobulinemia	2 (8.7%)
AST (IU/L)	49 [35-65]
ALT (IU/L)	48 [35-88]
γGT (IU/L)	127 [51-271]
ALP (IU/L)	102 [85-172]
Total bilirubin (µmol/L)	13.8 [10.0-17.0]
Creatinine (µmol/L)	119 [87-183]
eGFR CKD-EPI (mL/min)	52.5±29.2
eGFR MDRD (mL/min)	53.2±29.3
Albumin (g/L)	36.6±6.9
Hemoglobin (g/dL)	12.6±1.6
Platelet count (G/L)	156±82
Leukocytes (G/L)	6.1 [4.4-7.7]
INR	1.04 [1.00-1.09]
Prothrombin ratio (%)	94 [89-100]

Table 2: Antiviral therapy regimens

Regimen	
SOF+DCV	8 (34.8%)
SOF+DCV+RBV	3 (13.0%)
SOF+RBV	3 (13.0%)
SOF+PEG+RBV	1 (4.3%)
SOF+LEDI	3 (13.0%)
SOF+LEDI+RBV	3 (13.0%)
SOF+SIM	2 (8.7%)
Duration of therapy	
12 weeks	10 (43.5%)
24 weeks	11 (47.8%)
28 weeks	1 (4.3%)
32 weeks	1 (4.3%)
Initial dose	
SOF (mg/d)	400 [400-400]
DCV (mg/d)	60 [60-60]
LEDI (mg/d)	90 [90-90]
SIM (mg/d)	150 [150-150]
RBV (mg/d)	600 [400-800]
PEG (µg/w)	135 [135-135]

Table 3: Virological response during and after treatment according to the use of RBV

	Global (n=23)	RBV+ (n=10)	RBV- (n=13)	P
D0	23	10	13	
HCV RNA >= LLOQ	23 (100.0%)	10 (100.0%)	13 (100.0%)	*NA*
W2	19	8	11	
HCV RNA < LLOQ	6 (31.6%)	2 (25.0%)	4 (36.4%)	*p=1*
HCV RNA >= LLOQ	13 (68.4%)	6 (75.0%)	7 (63.6%)	
W4	22	9	13	
HCV RNA < LLOQ	14 (63.6%)	6 (66.7%)	8 (61.5%)	*p=1*
HCV RNA >= LLOQ	8 (36.4%)	3 (33.3%)	5 (38.5%)	
W8	22	10	12	
HCV RNA < LLOQ	22 (100.0%)	10 (100.0%)	12 (100.0%)	*NA*
W12	23	10	13	
HCV RNA < LLOQ	23 (100.0%)	10 (100.0%)	13 (100.0%)	*NA*
W24	13	7	6	
HCV RNA < LLOQ	13 (100.0%)	7 (100.0%)	6 (100.0%)	*NA*
EOT	23	10	13	
HCV RNA < LLOQ	23 (100.0%)	10 (100.0%)	13 (100.0%)	*NA*
FUW4	23	10	13	
HCV RNA < LLOQ	22 (95.7%)	10 (100.0%)	12 (92.3%)	*p=1*
HCV RNA >= LLOQ	1 (4.3%)	0 (0.0%)	1 (7.7%)	
FUW12	23	10	13	
HCV RNA < LLOQ	22 (95.7%)	10 (100.0%)	12 (92.3%)	*p=1*
HCV RNA >= LLOQ	1 (4.3%)	0	1 (7.7%)	

Table 4: Safety profile of antiviral therapy according to RBV use

Variables	Global (n=23)	RBV+ (n=10)	RBV- (n=13)	P
AE				
Yes	20 (87.0%)	10 (100.0%)	10 (76.9%)	*p = 0.23*
No	3 (13.0%)	0 (0.0%)	3 (23.1%)	
SAE				
Yes	9 (39.1%)	4 (40.0%)	5 (38.5%)	*p = 1*
No	14 (60.9%)	6 (60.0%)	8 (61.5%)	
AE Grade ¾				
Yes	11 (47.8%)	4 (40.0%)	7 (53.8%)	*p=0.68*
No	12 (52.2%)	6 (60.0%)	6 (46.2%)	
Acute rejection				
No	23 (100.0%)	10 (100.0%)	13 (100.0%)	*NA*
Anaemia				
Grade 0	1 (4.3%)	1 (10.0%)	0 (0.0%)	
Grade 1/2	6 (26.1%)	4 (40.0%)	2 (15.4%)	*p = 0.036*
Grade 3/4	4 (17.4%)	3 (30.0%)	1 (7.7%)	
No	12 (52.2%)	2 (20.0%)	10 (76.9%)	
Lowest Hb value (g/dl)	10.4 ± 2.2	9.1 ± 1.7	11.3 ± 2	*p = 0.009*
EPO use				
Yes	9 (39.1%)	7 (70.0%)	2 (15.4%)	*p = 0.013*
No	14 (60.9%)	3 (30.0%)	11 (84.6%)	
RBC transfusion				
Yes	4 (17.4%)	4 (40.0%)	0 (0.0%)	*p = 0.023*
No	19 (82.6%)	6 (60.0%)	13 (100.0%)	
RBV management				
Reduction	6 (26.1%)	6 (60.0%)	-	*NA*
Withdrawal	3 (13.0%)	3 (30.0%)	-	*NA*
Neutropenia				
Grade 1/2	2 (8.7%)	1 (10.0%)	1 (7.7%)	
Grade 3/4	1 (4.3%)	1 (10.0%)	0 (0.0%)	*p = 0.7*
No	20 (87.0%)	8 (80.0%)	12 (92.3%)	
Thrombopenia				
Grade ½	7 (30.4%)	3 (30.0%)	4 (30.8%)	*p = 1*
No	16 (69.6%)	7 (70.0%)	9 (69.2%)	
Cardiovascular event				
No	23 (100.0%)	10 (100.0%)	13 (100.0%)	*NA*
Infection				
Grade 1/2	6 (26.1%)	4 (40.0%)	2 (15.4%)	
Grade 3/4	3 (13.0%)	1 (10.0%)	2 (15.4%)	*p = 0.48*
No	14 (60.9%)	5 (50.0%)	9 (69.2%)	

Figure 1

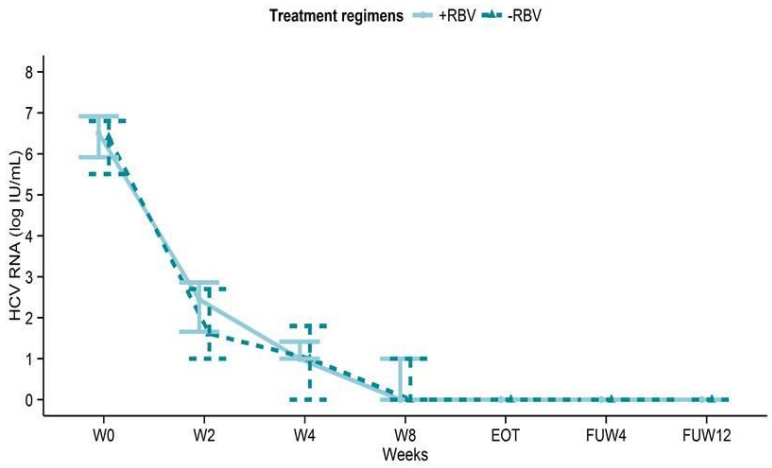

Figure 2

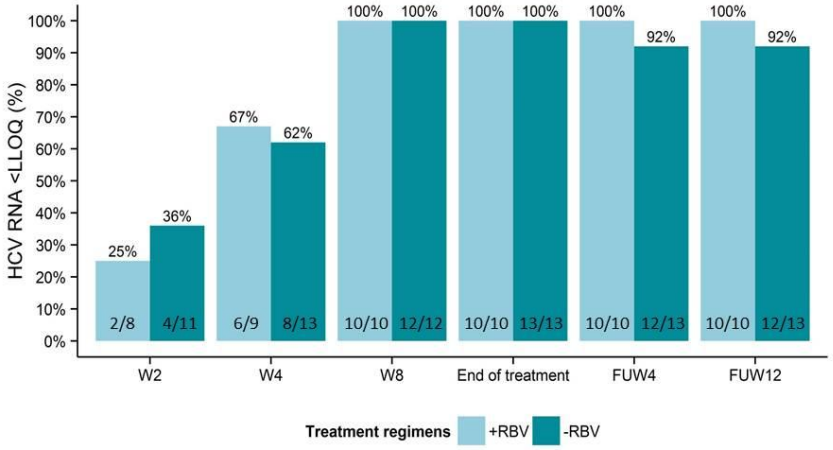

Figure 3

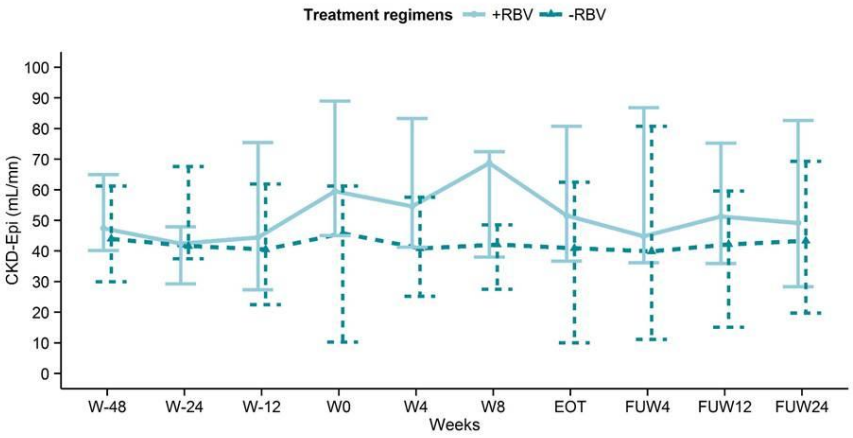

Figure 4

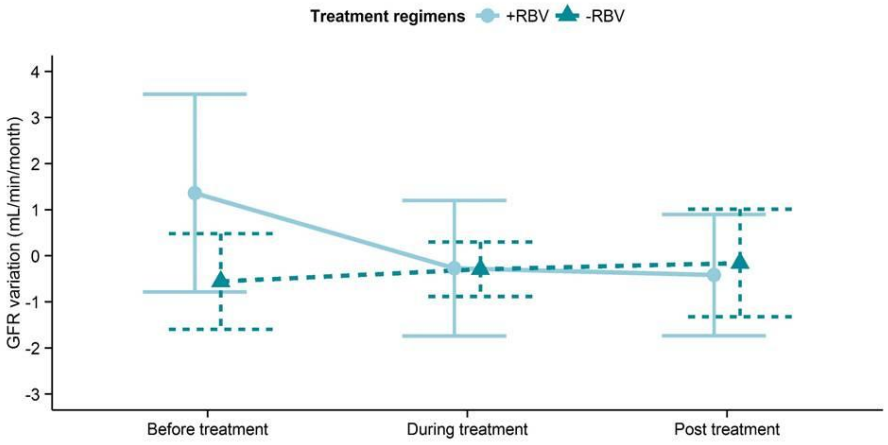

Figure 5